Systems for Identifying New Drugs Are Often Faulty

Screening potential anticancer drugs sounds easy. Just take a candidate drug, add it to a tumor type of choice, and then monitor whether the agent kills the cells or inhibits cancer growth. Too bad it hasn't been that simple. Even as investigators try to develop a new generation of more effective and less toxic anticancer drugs that directly target the gene changes propelling cells toward uncontrollable division (see p. 1036), they face a long-standing problem: sifting through potential anticancer agents to find ones promising enough to make human clinical trials worthwhile.

Indeed, since formal screening began in 1955, many thousands of drugs have shown activity in either cell or animal models, but only 39 that are used exclusively for chemotherapy, as opposed to supportive care, have won approval from the U.S. Food and Drug Administration. "The fundamental problem in drug discovery for cancer is that the model systems are not predictive at all," says Alan Oliff, executive director for cancer research at Merck Research Laboratories in West Point, Pennsylvania.

Pharmaceutical companies often test drug candidates in animals carrying transplanted human tumors, a model called a xenograft. But not only have very few of the drugs that showed anticancer activity in xenografts made it into the clinic, a recent study conducted at the National Cancer Institute (NCI) also suggests that the xenograft models miss effective drugs. The animals apparently do not handle the drugs exactly the way the human body does. And attempts to use human cells in culture don't seem to be faring any better, partly because cell culture provides no information about whether a drug will make it to the tumor sites.

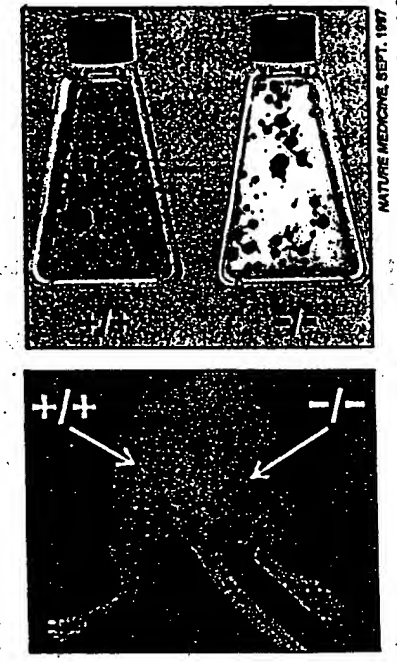
The pressure is on to do better. So researchers are now trying to exploit recent discoveries about the subtle genetic and cellular changes that lead a cell toward cancer to create cultured cells or animal models that accurately reproduce these changes. "The real challenge for the 1990s is how to maximize our screening systems so that we are using the biological information that has accumulated," says Edward Sausville, associate director of the division of cancer treatment and diagnosis for the developmental therapeutics program at the NCI. "In short, we need to find faithful representations of carcinogenesis."

The first efforts to do so date back to the end of World War II, when hints began emerging that some chemicals might have cancer-fighting effects. That evidence encouraged many chemists to explore the anticancer potential of similar agents shelved in their laboratories. And after commercial interests decided against helping the academics set up an efficient way to screen their chemicals, the NCI stepped in.

The institute started by pulling together mouse models of three tumors: a leukemia, which affects blood cells; a sarcoma, which arises in bone, muscle, or connective tissue; and a carcinoma, the most common type of cancer, which arises in epithelial cells and includes such major killers as breast, colon, and lung cancers. Initially, many of the agents tested in these models appeared to do well. However, most worked against blood cancers such as leukemia and lymphoma, as opposed to the more common solid tumors. And when tested in human cancer patients, most of these compounds failed to live up to their early promise.

Researchers blamed the failures on the fact that the drugs were being tested against mouse, not human, tumors, and beginning in 1975, NCI researchers came up with the xenograft models, in which investigators implant human tumors underneath the skin of mice with faulty immune systems. Because the animals can't reject the foreign tissue, the tumors usually grow unchecked, unless stopped by an effective drug. But the results of xenograft screening turned out to be not much better than those obtained with the original models, mainly because the xenograft tumors don't behave like naturally occurring tumors in humans—they don't spread to other tissues, for example. Thus, drugs tested in the xenografts appeared effective but worked poorly in humans. "We had basically discovered compounds that were good mouse drugs rather than good human drugs," says Sausville.

The xenograft models may also have



Not a matched pair. In the clonogenic assay *(top)*, tumor cells with (+/+) and without (–/–) the *p21* gene responded similarly to radiation. But in mice, the *p21*– tumors often shrank, while those having the gene never did.

cer agents currently used in patients against 48 human cancer cell lines transplanted individually into mice, they found that 30 of the tumors did not show a significant response—defined as shrinking by at least 50%—to any of the drugs.

Researchers have not yet figured out why so many of the xenografts were insensitive to the drugs. But the NCI team says that the result means that drugs would have to be screened against six to 12 different xenografts to make sure that no active anticancer drugs were missed. That's an expensive proposition, as the average assay costs about $1630 when performed by the government and $2900 when done commercially. "I cannot get on my pulpit and say that the way we are doing this is the best way, because I don't think there is a good way to do it," says Sausville.

To create better models of cancer development in humans, investigators are now drawing on the growing knowledge f human cancer-related gene mutations. They are genetically altering mice so that they carry the same kinds of changes—either abnormal activation of cancer-promoting oncogenes or loss of tumor-suppressor g nes—that lead to cancer in humans. The hope is that the mice will develop tumors that behave the same way the human tumors do.

So far, the results from these mouse models have been mixed, however. One mutant mouse strain, for example, lacks a working *APC* gene, a tumor suppressor that leads to colon cancer when lost r inactivated. This mouse seems to do well at re-creating the early signs of colon cancer. But in the later stages of the disease, the type of mutations in the tumors begin to diverge from those in human colon cancer, and the disease manifests itself differently as well. It spares the liver, for example, unlike the human cancer.

Other new mouse models have fared even worse. Take the one in which the *retinoblastoma* (*RB*) tumor-suppressor gene was knocked out. In humans, loss of *RB* leads to a cancer in the retina of the eye. But when the gene is inactivated in mice, the rodents get pituitary gland tumors. And *BRCA1* knock-

outs—which are supposed to simulate human breast and ovarian cancer—don't get any tumors at all. "One might expect that these animals would also mimic human symptoms, not just the genetic mutations," says molecular biologist Tyler Jacks of the Massachusetts Institute of Technology. "In fact, that is usually the exception, not the rule."

Why gene knockouts in mice have effects so different from those of the corresponding mutations in humans is unclear. One possibility is that in mice, other genes can compensate for a missing gene, such as BRCA1. Another, says Jacks, is that "the genetic wiring for growth control in mice and humans is subtly different."

The limitations of animal models have spurred the NCI, among others, to test drug candidates in cultures of human cells. The institute now relies on a panel of 60 human tumor cell lines, including samples of all the major human malignancies. Drugs to be tested are fed to subsets of the panel, based on tumor cell type, and their cell-killing activity is monitored.

Over the last 7 years, the panel has been used to screen almost 63,000 compounds, and 5000 have exhibited tumor cell-killing activity. But that has created another dilemma, because so many compounds show antitumor cell activity in culture, and the cost of bringing them all to clinical trials—where most don't work anyway—would be daunting. As Sausville asks: "How do you prioritize so many compounds for clinical trials?" For that, the NCI uses a computer database to sift through past antitumor agents and look for only those compounds with novel mechanisms of action. Computer screening has whittled the number of promising agents down to about 1200, according to Sausville.

Those compounds are then tested in what is known as a hollow fiber model, in which tiny tubes filled with tumor cells are implanted into mice in a variety of sites. By monitoring the tumor cell–killing effects of drugs on the implants, researchers can test which drugs actually make it to the tumor sites when the drugs are administered in different ways: intravenously versus orally, for example. Sausville cautions, however, that it's still too early to tell how predictive these screens are, because only a few of the drugs tested have gone far enough to show efficacy in humans.

Both drug screeners and doctors also use another cell culture method, the so-called clonogenic assay, to sift through potential anticancer drugs. They grow cell lines or a patient's tumor cells in petri dishes or culture flasks and monitor the cells' responses to various anticancer treatments. But clonogenic assays have their problems, too. Sometimes they don't work because the cells simply fail to divide in culture. And the results cannot tell a researcher how anticancer drugs will act in the body.

What's more, new results from Bert Vogelstein's group at Johns Hopkins University School of Medicine add another question mark about the assay's predictive ability. Todd Waldman, a postdoc in the Vogelstein laboratory, found that xenografts and clonogenic assays deliver very different messages about how cancer cells lacking a particular gene, *p21*, respond to DNA-crippling agents.

TESTING THE XENOGRAFT ASSAY

Type of Cancer	Cell Lines Tested	% of Tumors Responding to Drugs: Minimal Response (< 40% shrinkage)	Significant Response (> 50% shrinkage)
Colon	9	31%	5%
Lung, non–small cell	7	49%	8%
Breast	6	51%	19%
Ovarian	3	35%	0
Renal	6	43%	0

Radiation, like many of the drugs used to treat cancer, works by damaging the cells' DNA. This either brings cell replication to a halt or triggers a process known as apoptosis in which the cells essentially commit suicide. Waldman wanted to see how *p21*, one of the genes involved in sensing the DNA damage and halting cell replication, influences that response to radiation.

In the mouse xenograft assay, Waldman and his colleagues found that the radiation cured 40% of the tumors composed of cells lacking *p21*, while tumors made of cells carrying the gene were never cured. But this difference was not apparent in the clonogenic assay, where the radiation appeared to thwart the growth of both dispersed tumor cell types. "We showed this gross difference in sensitivity in real tumors in mice and in the clonogenic assay," Waldman says.

He suggests that the different responses in the two systems have to do with the fact that a subset of *p21* mutants die in response to radiation, while cells with the normal gene merely arrest cell division. Either way, the dispersed tumor cells in the clonogenic assay will fail to grow. However, in the xenograft tumors, which consist of many cells in a solid mass, the arrested, but nonetheless living, *p21*$^+$ tumor cells may release substances that encourage the growth of any nearby tumor cells that escaped the effects of the radiation. But tumor cells lacking the *p21* gene die, and because dead cells cannot "feed" neighboring

The finding indicates that the clonogenic assay can't always predict how a tumor will respond to a drug in an animal. Still, by linking the different responses in two models to the presence or absence of a specific gene system, the Waldman team's results help clarify why tumor cells might respond differently in culture and in animals. Indeed, the general idea that a tumor's drug sensitivity may be linked to the genetic mutations it carries has led others to try to use cells with comparable mutations to identify better chemotherapeutic agents.

Leland Hartwell, Stephen Friend, and their colleagues at the Fred Hutchinson Cancer Research Center in Seattle are pioneering one such effort. They are building on previous work in which Hartwell's team discovered a series of yeast genes, called checkpoint genes, that normally stop cells from progressing through the cell cycle and dividing if they have abnormalities such as unrepaired DNA damage. Because mutations in checkpoint and other cell cycle–related genes have been linked to human cancers, looking for drugs that restore normal growth control in mutated yeast might be one way to find new cancer therapies (see Article on p. 1064).

The NCI is taking a similar tack. They are looking to see if they can reclassify the cells in their panel, which was set up based on tissue type—breast cancer versus colon cancer, for example—according to the types of genetic defects the cells carry. To enable drugs that counteract specific defects to be prescribed most effectively, researchers are also developing technologies for analyzing the gene defects in each patient's tumors. That way, if drugs that correct specific defects can be identified, they could then be matched to each individual's tumor cell makeup. "This would be so valuable," says Homer Pearce, vice president of cancer research and clinical investigation at Eli Lilly and Co. in Indianapolis. "It would help to identify patients that have the greatest chance of benefiting from therapy, while minimizing the number that would be exposed to a treatment that would not work."

Indeed, Merck's Oliff says, "the future of cancer drug screening is turning almost exclusively toward defining molecular targets." If the approach works, drug developers would finally have an easy way to identify promising cancer drugs, and cancer patients might have an array of new treatments.

–Trisha Gura

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